WHAT IS CLAIMED:

1. A method for inserting a nucleic acid fragment into a circular vector, which comprises:

(a) stably joining an insertion end of a nucleic acid fragment with an insertion end of a linearized vector at a first nucleic acid concentration under conditions favoring intermolecular joining, to form a linear vector-insert concatemer;

(b) melting hybridized cohesive circularization ends in said vector-insert concatemer to form a linear vector-insert monomer having single-stranded cohesive circularization ends; and

(c) reannealing said single-stranded cohesive circularization ends at a second nucleic acid concentration under conditions favoring circularization to form a circularized vector containing a nucleic acid insert;

wherein said second nucleic acid concentration is more dilute than said first nucleic acid concentration and wherein said cohesive circularization ends are between about 8 and about 50 nucleotides in length.

2. A method for inserting a nucleic acid fragment into a circular vector, which comprises:

(a) stably joining an insertion end of a nucleic acid fragment with an insertion end of a linearized vector at a first nucleic acid concentration under conditions favoring intermolecular joining, to form a linear vector-insert construct with complementary circularization ends, wherein one or both circularization ends of said vector-insert construct (1) are attached to an enzyme or enzyme complex capable of

covalently joining DNA ends, and (2) are blocked from covalent joining;

(b) unblocking said circularization ends of said vector-insert construct; and

(c) joining said circularization ends of said insert-vector construct at a second nucleic acid concentration in an intramolecular reaction mediated by said enzyme or enzyme complex under conditions favoring circularization, to form a circularized vector containing a nucleic acid insert;

wherein said second nucleic acid concentration is more dilute than said first nucleic acid concentration circularization.

3. The method of Claim 2 wherein:

said enzyme or enzyme complex is a site-specific topoisomerase that is covalently linked through a 3' phosphate to a circularization end;

one or both circularization ends are blocked from covalent joining by 5' phosphates;

said unblocking is achieved by removing said 5' phosphates from said circularization ends; and

said site-specific topoisomerase does not substantially covalently join said circularization ends of said vector-insert construct until the 5'- phosphates are removed from said circularization ends.

4. The method of Claim 1 wherein said linearized vector comprises two vector parts, each vector part having a cohesive circularization end which can hybridize to a complementary cohesive circularization end of the second vector part and an insertion end.

5. The method of Claim 1 wherein said linearized vector comprises an insertion end and a cohesive circularization end and wherein said nucleic acid fragment comprises a complementary insertion end and a complementary cohesive circularization end.

6. The method of Claim 1 wherein:

said vector has a recognition site for an enzyme or enzyme complex which creates a first nick in one strand which is about 8 to about 50 nucleotides from a second nick in the other strand;

after said joining, said method further comprises nicking said vector-insert concatemer with said enzyme or enzyme complex to produce cohesive circularization ends;

said nicking is not accompanied by packaging into phage particles; and said recognition site is at least about 15 nucleotides in length.

7. The method of Claim 2 wherein said linearized vector comprises two vector parts, each vector part having an insertion end and a circularization end, and wherein one or both of said circularization ends (1) are attached to an enzyme or enzyme complex capable of covalently joining DNA ends, and (2) are blocked from covalent joining.

8. The method of Claim 2 wherein:

said linearized vector comprises an insertion end and a circularization end;

said nucleic acid fragment comprises a complementary insertion end and a complementary circularization end;

either said circularization end or said complementary circularization end is attached to an enzyme or enzyme complex capable of covalent joining DNA ends; and

both said circularization end and said complementary circularization end are blocked from covalent joining.

9. The method of Claim 1 wherein said cohesive circularization ends are formed by an enzyme or enzyme complex which creates a first nick in one strand which is about 8 to about 50 nucleotides from a second nick in the other strand.

10. The method of Claim 1 wherein said cohesive circularization ends are formed by a terminase of a bacteriophage or virus, but wherein said vector-insert monomer is not packaged into a phage particle.

11. The method of Claim 1 wherein said cohesive circularization ends are formed by ligation of oligonucleotide adapters, tailing with terminal transferase, digestion with an exonuclease, digestion with a DNA polymerase possessing proofreading activity, or removal of uracil residues by uracil DNA glycosylase after polymerase chain reaction using dUMP-containing primers.

12. The method of Claim 1 wherein said cohesive circularization ends comprise nucleotide analogs.

13. The method of Claim 1 or 2 wherein said joining is mediated by ligase and wherein ligase does not substantially covalently join said circularization ends.

14. The method of Claim 1 or 2 wherein said joining is mediated by a site-specific topoisomerase covalently linked to said insertion end of said linearized vector or said insertion end of said nucleic acid fragment.

15. The method of Claim 1 or 2 wherein said joining is mediated by Vaccinia virus topoisomerase I or a Vaccinia virus topoisomerase I fusion protein covalently linked to said insertion end of said linearized vector or said insertion end of said nucleic acid fragment.

16. The method of Claim 1 or 2 wherein at least one of said insertion ends or at least one of said circularization ends comprises a blunt end covalently linked to a site-specific topoisomerase and wherein said blunt end is prepared by:

(a) creating a nick in a DNA strand that is exactly opposite to a topoisomerase cleavage site in the complementary DNA strand; and

(b) cleaving with said site-specific topoisomerase at said topoisomerase cleavage site to produce a blunt end.

17. The method of Claim 1 or 2 wherein at least one of said insertion ends or at least one of said circularization ends comprises a 3' overhang covalently linked to a site-specific topoisomerase and wherein said 3' ~~overhand~~ overhang is prepared by:

(a) creating a nick in a DNA strand that is located one or more nucleotides in the 3' direction from a position exactly opposite to a topoisomerase cleavage site in the complementary DNA strand; and

(b) cleaving with said site-specific topoisomerase at said topoisomerase cleavage site to produce a 3' overhang.

18. The method of Claim 2 wherein said joining is mediated by annealing a cohesive insertion end of said linearized vector to a complementary cohesive insertion end of said nucleic acid fragment, wherein each of said cohesive insertion ends is between about 8 to about 50 nucleotides in length.

19. The method of Claim 1 wherein said reannealing is performed at a salt concentration which is higher than the salt concentration used for said melting.

20. The method of Claim 1 wherein said reannealing is performed at a salt concentration which is between about 100 mM and about 7.5 M.

21. The method of Claim 1 wherein said reannealing is performed at about 50° C to about 85° C.

22. The method of Claim 1 or 2 wherein said nucleic acid fragment is selected from the group consisting ~~essentially~~ of eukaryotic, prokaryotic, viral, and bacteriophage genomic DNA, cDNA, cDNA:RNA hybrid, polymerase chain reaction product and vector DNA.

SUB A1 23. The method of Claim 1 or 2 wherein said first nucleic acid concentration can comprise about 10^{-21} to about 10^{-14} mole nucleic acid fragment.

24. The method of Claim 1 ~~or~~ 2 wherein said first nucleic acid concentration comprises a molar ratio of linearized vector to nucleic acid fragment which is about 10:1 to about 100,000,000:1.

25. The method of claim 1 or 2 wherein said conditions favoring intermolecular joining are macromolecular crowding conditions.

26. The method of Claim 1 or 2 wherein said second nucleic acid concentration is less than one tenth of said first nucleic acid concentration.

27. The method of Claim 1 or 2 wherein the efficiency of insertion of said nucleic acid fragment into said circular vector is at least about 95%.

28. The method of Claim 1 or 2 wherein the efficiency of forming a circularized vector containing only one nucleic acid insert is at least about 95%.

29. The method of Claim 1 or 2 wherein the efficiency of forming a circularized vector containing an insert is substantially the same over a range of insert sizes varying from about 20 base pairs to about 20,000 base pairs.

30. The method of Claim 1 or 2 wherein said nucleic acid fragment is between 20 base pairs and 100,000 base pairs in length.

31. A nucleic acid insert in a circular vector prepared by the method of Claim 1 or 2.

32. A genomic or cDNA library in a circular vector prepared by the method of Claim 1 or 2.

33. A mixture formed from a joining reaction of a population of linearized vectors with a population of nucleic acid fragments, wherein at least 90% 95% of said nucleic acid fragments are inserted into circularized vectors and wherein at least 95% of said circularized vectors contain only one nucleic acid fragment insert.

34. The mixture of Claim 33 or 65 wherein said population of nucleic acid fragments is cDNA or genomic DNA.

35. A population of prokaryotic or eukaryotic host cells comprising the mixture of Claim 33.

36. A population of circularized vectors containing nucleic acid fragment inserts which is isolated from said population of prokaryotic or eukaryotic host cells of Claim 35.

37. A linearized vector comprising an origin of replication, an insertion site, and two complementary cohesive circularization ends, wherein:

each of said cohesive circularization ends is at least about 20 base pairs from said insertion site;

said cohesive circularization ends are between about 8 and about 50 nucleotides in length; and

upon hybridization ligase does not substantially covalently join said cohesive circularization ends.

38. The linearized vector of Claim 37 wherein said vector is cleaved in said insertion site with at least one restriction enzyme and dephosphorylated.

39. A linearized vector comprising an origin of replication, a blunt or short sticky insertion end, and a cohesive circularization end, wherein said short sticky insertion end is between 1 and 7 nucleotides in length and said cohesive circularization end is between about 8 and about 50 nucleotides in length.

40. A kit comprising a first compartment containing the linearized vector of any one of Claims ~~37-39~~.

41. The kit of Claim 40 which further comprises:

a second compartment containing a DNA ligase;

a third compartment containing a buffer comprising polyethylene glycol of high molecular weight; and

a fourth compartment containing a buffer comprising a salt.

42. A linearized vector comprising an origin of replication, two blunt or sticky ends, and two cohesive ends, wherein:

said cohesive ends are between about 8 and about 50 nucleotides in length;

each of said blunt or sticky ends is covalently linked to a site-specific topoisomerase; and

each of said blunt or sticky ends has a 5'-phosphate.

43. A linearized vector comprising an origin of replication, a blunt or sticky end covalently linked to a site-specific topoisomerase, and a cohesive end, wherein said cohesive end is between about 8 and about 50 nucleotides in length.

44. A kit comprising a first compartment containing the linearized vector of Claim 42 or 43.

45. The kit of Claim 44 which further comprises:

a second compartment containing a buffer comprising polyethylene glycol of high molecular weight; and

a third compartment containing a buffer comprising a salt.

46. A linearized vector comprising an origin of replication, two insertion ends, and two circularization ends wherein:

each of said circularization ends is located at least 15 base pairs from each of said insertion ends;

each of said insertion ends is covalently linked to a site-specific topoisomerase;

one or both of said circularization ends are covalently linked to a site-specific topoisomerase; and

each of said insertion ends and each of said circularization ends has a 5'-phosphate.

47. A kit comprising a first compartment containing the linearized vector of Claim 46.

48. A linearized vector comprising an origin of replication, a bacteriophage or virus cos site, and two insertion ends covalently linked to a site-specific topoisomerase.

49. A kit comprising a first compartment containing the linearized vector of Claim 48.

50. The kit of Claim 49 which further comprises:

a second compartment containing a buffer comprising high molecular weight polyethylene glycol;

a third compartment containing a terminase; and

a fourth compartment containing a buffer comprising a salt.

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